SHORT COMMUNICATION article

Prevalence of gram-positive cocci in the oral cavity of Libyan smoking students at the Faculty of Medical Technology, Sabha University

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Abstract: Oral microbial communities are in direct contact with tobacco smoking, which may affect these communities. This study aimed to investigate whether tobacco smoking alters the microbial diversity in oral fluid, with a focus on Gram-positive bacteria. Forty samples were collected from students at the Faculty of Technology at Sabha University, Libya during 2025. The participants were then divided into two groups: Group 1: Smokers and Group 2: Non-smokers. All the smokers sampled were found to be infected, while the non-smoker students did not have the infection or the type of bacteria required for the study. Two types of bacteria were found in the smokers: type 1 (*Streptococcus*) and type 2 (*Staphylococcus*). More people were infected with type 1 than with type 2. Among type 1 smokers, nine smokers did not suffer from dental caries, while five smokers had dental caries and were infected. These findings confirm that tobacco smoking significantly affects Gram-positive salivary microbes.

Introduction

Saliva is a biological fluid secreted by salivary glands in the oral cavity [1]. The oral cavity contains a significant bacterial diversity that is specific to each individual and shows long-term stability over the years [2, 3]. In particular, oral fluid consists of some Gram-positive bacteria involved in oral cavity infections, and infections distant from the oral cavity, such as infective endocarditis [2]. Many oral disorders and their treatment may have an impact on the bacterial diversity of the salivary microbiota [2]. Some external factors may influence microbial diversity in the salivary microbiota. Among these external factors, cigarette smoke and tobacco smoking have been shown to affect the oral microbiota [4, 5]. The microbial communities in the mouth and the nose have direct contact with cigarette smoke and may thus be affected by it. Cigarette smoke contains numerous toxicants to which smokers are regularly exposed on a periodic basis. These toxicants can potentially perturb the microbial ecology of the mouth via antibiotic effects, oxygen deprivation, or other potential mechanisms [6]. Cigarette smoke contains over 1,200 harmful chemicals that accumulate in the body, overwhelming its natural defense and excretion mechanisms and leading to widespread health damage. Among the most dangerous are nicotine, carbon monoxide, tar, hydrogen cyanide, ammonia, and phenol. Collectively, these chemicals weaken the immune system, making the body more susceptible to infections and chronic diseases [7].

Bacteria are microscopic single-celled organisms found everywhere, most of which are harmless or beneficial, though a few can cause disease [8]. Gram-positive bacteria include cocci, such as *Staphylococcus* (clusters, catalase-positive) and *Streptococcus* (chains, catalase-negative, with several groups), and bacilli, which may be spore-forming (Bacillus, Clostridium), non-spore-forming (Listeria, Corynebacterium), or filamentous (Nocardia, Actinomyces) [9]. *Streptococcus pneumoniae* is a Gram-positive, lancet-shaped bacterium and the leading cause of community-acquired pneumonia, especially in winter and spring [10]. *Streptococcus pyogenes* are Gram-positive, chain-forming group A streptococci that show β-hemolysis on blood agar and cause diseases such as pharyngitis, scarlet fever, impetigo, and cellulitis [11, 12]. *Staphylococci* are Gram-positive cocci forming clusters, with *Staphylococcus aureus* being the most pathogenic, causing skin, deep tissue, hospital-acquired, and toxin-mediated infections. The growth and toxin production of *Staphylococcus aureus* are shaped by environmental and nutritional conditions, including sugar and protein sources, temperature, and pH. While the bacteria are heat-sensitive, their toxins resist heat. They can tolerate high salt, low water activity, and some toxic substances, though growth is influenced by competing microorganisms and radiation exposure [13]. Therefore, this study was designed to compare oral bacteria in smoking and non-smoking students and to examine the potential effect of tobacco smoking on oral Gram-positive bacteria in general.

Materials and methods

Study design: Forty samples were collected from male students (age: 18-23 years) at the Faculty of Technology, Sabha University, during May 2025. The samples were then divided into two groups: Smokers (n=19) and Non-smokers (n=21). Samples were taken from oral swabs using a sterile cotton swab according to standard aseptic methods. The sample was taken by moving it around the cheeks and gums for 30 secs. Contamination of the swab with saliva was avoided. The samples were subjected to microscopic examination and culture. Organisms were identified using standard microbiological techniques. Gram-positive bacteria were isolated from the oral cavity of smokers and nonsmokers. Before swab collection, the inside of the mouth was examined for any inflammation or the presence of any membranous secretions or pus.

Data collection: An oral swab was collected. Before collecting the swab, the inside of the mouth was examined for inflammation or the presence of any membrane exudates or pus. Using a sterile cotton swab, the sample was taken by swirling it around the cheeks and gum for 30 sec. The contamination of the swab with saliva was avoided. The swab stick was transported within one hour of collection to the laboratory for analysis. The swab sticks were aseptically placed in peptone water broth, which was incubated at 37°C for 24 hrs. and was used as stock.

Culture: A loopful of stock was streaked on Columbia agar containing colistin and nalidixic acid, incubated at 37^0C for 24 hrs. under aerobic conditions. Colonies were sub-cultured and isolated on COS-ANC medium (one COS-ANC culture dish for each transplanted colony) and incubated at 37^0C for 24 hrs. in order to obtain a pure culture. This protocol was designed to isolate Gram-positive bacteria and avoid contamination by other bacteria forming the oral microbiota [4] and was observed microscopically (Gram staining) and biochemically (coagulase, catalase, oxidase, and urease).

Ethical approval: All the participants provided verbal and written consent on their participation in the trial and their right to withdraw at any time, in accordance with the ethical guidelines outlined in the 1975 Declaration of Helsinki. In addition, the study was approved by the University Ethics Committee (Sebha-15-2025).

Statistical analysis: The Chi-square test and Fisher's test were used to confirm the association between the groups. A p-value of less than 0.05 was considered statistically significant.

Results and discussion

The present data indicated that a high frequency of participants did not suffer from tooth decay, compared to those who reported suffering from it (**Figure 1**). This reflects a relatively positive indicator of oral health among students at Sabha University, Libya. This may be due to acceptable health awareness or environmental and nutritional factors [14]. However, the fact that a quarter of the sample suffered from tooth decay calls for a deeper study of behavioral patterns and influencing factors. This finding is consistent with the published study indicating that tooth decay is a major public health problem worldwide [15]. According to the previous data, tooth decay remains one of the most common non-communicable oral diseases, affecting both children and adults [15]. Approximately two billion people suffer from permanent tooth decay, while 510 million children suffer from baby tooth decay [16]. A recent study suggests that the composition of the oral microbiome (e.g., balance between cariogenic and non-cariogenic bacteria) is altered by smoking [17]. For instance, smokers tend to have a higher abundance of cariogenic microbes in supragingival plaque [18]. Also, the present findings showed that more than half of the participants suffered from dry mouth (xerostomia) (**Figure 2**). This is an indication of a possible salivary dysfunction, which could affect the oral microbial balance [19]. This may be linked to smoking habits or the use of certain medications. Studies have reported a strong association between smoking and xerostomia. For example, a case-control, cross-sectional study conducted by Kakoe and others [20] showed that the prevalence of dry mouth was 40.0% in smokers. We suggest that xerostomia requires more awareness regarding oral hydration and maintaining a healthy oral environment.

In **Figure 3**, all the participants reported not having had braces in recent years. This reflected either a lack of medical need or a lack of interest in this type of dental care. It may indicate limited awareness or financial resources. The importance of correcting malocclusions and their impact on oral health should be highlighted. The results showed that about half of the sample did not use toothpaste, compared to the remaining who did (**Figure 4**). This low rate of toothpaste use is concerning from a preventive perspective, as toothpaste contains antibacterial and enamel-strengthening compounds [14, 15]. This highlights the need to strengthen awareness programs on the importance of oral hygiene [14, 15]. Another study supports the current findings that non-use of toothpaste is a strong risk factor for dental caries, enamel demineralization, and erosion, and indirectly for gingival and periodontal diseases [21]. The current data showed that 90.0% of the participants did not use the Miswak, compared to the rest who regularly used it (**Figure 5**). Despite the proven health benefits of the Miswak, its use remains low. This has been attributed to a decline in traditional practices or a lack of awareness of its benefits. Many studies confirmed that low Miswak use is common in many communities [17, 22, 23]; however, a study showed that only 8.0% Miswak users were in the Aseer Region study. Another study showed that Miswak has demonstrable oral health benefits when used properly, especially as an adjunct to toothbrushing [14, 15, 23]. 95.0% of the participants reported not using mouthwash, while 5.0% did (**Figure 6**).

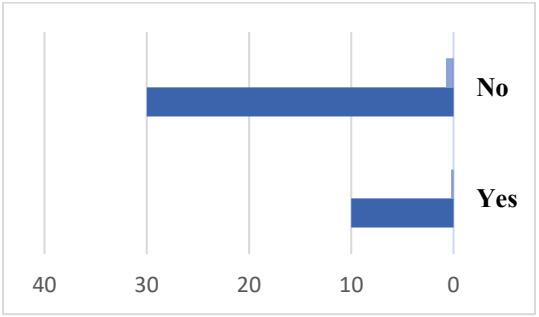

Figure 1: Distribution of Libyan individuals with and without dental caries

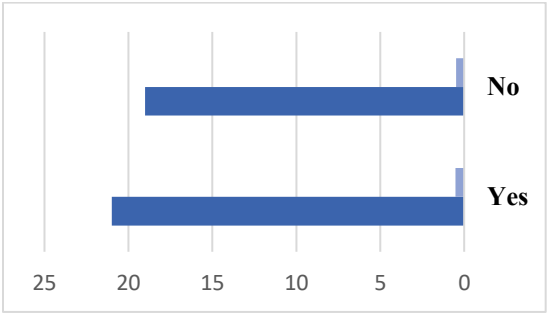

Figure 2: Distribution of Libyan individuals with and without dry mouth

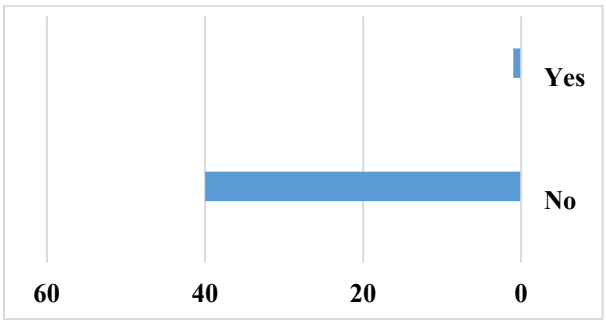

Figure 3: Distribution of braces users and non-braces users

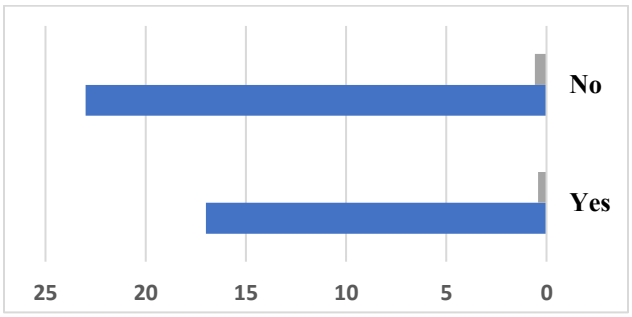

Figure 4: Distribution of Libyan individuals using and not using toothpaste

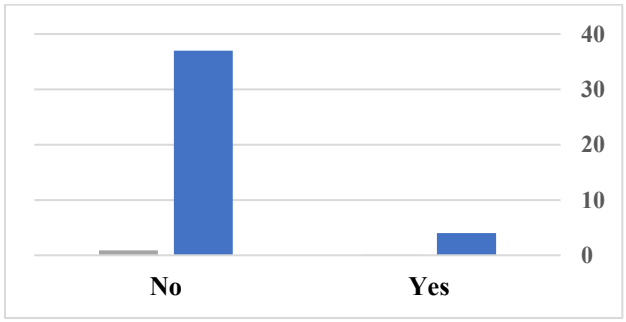

Figure 5: Distribution of Libyan individuals who use and do not use the miswak

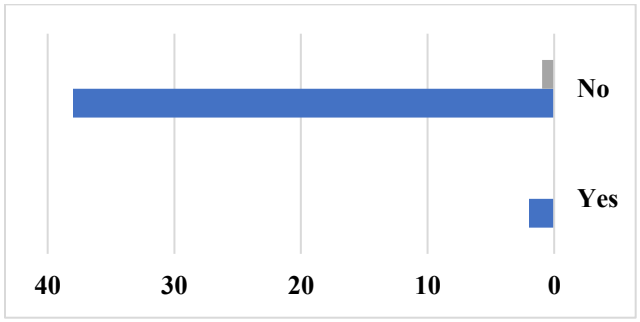

Figure 6: Distribution of Libyan individuals using and not using mouthwash

These results reflect the low uptake of this important preventive product, which helps reduce bacterial buildup and improve oral odor [24]. Ren and others [25] support the current findings that mouthwash is important for prevention, as it demonstrated clear benefits of mouthwash (chlorhexidine, essential oils, and propolis) for reducing plaque, gingivitis, and in some cases oral bacterial counts [25]. The current results showed that 42.5% suffer from bad breath (halitosis), 52.5% do not, and 5.0% experience it occasionally (**Figure 7**). These findings reflect the presence of an existing problem among a significant proportion of students. This problem may be caused by poor oral hygiene, systemic factors, or bacteria in their study [24]. Dey and others [26] reported that half of the participants had self-perceived halitosis, which was high among smokers and those who did not use toothpaste, used mouthwash infrequently, had dry mouth, food accumulation, and poor oral hygiene habits [24, 26]. The findings indicated that 90.0% of the sample did not take antibiotics, compared to only 10.0% who used them (**Figure 8**). This is a good indicator in terms of reducing antibiotic resistance [27]. It reflected a potential awareness of the importance of avoiding overuse. However, it is important to ensure that those who require antibiotics receive them correctly and appropriately.

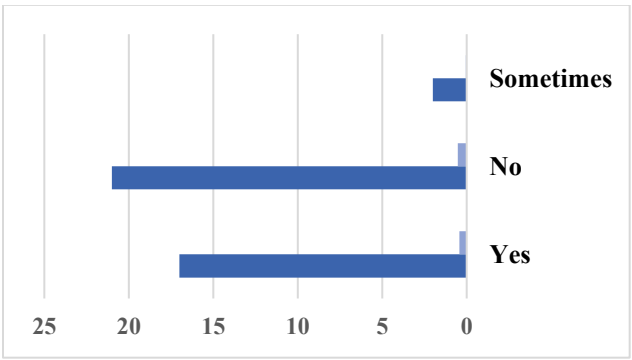

Figure 7: Distribution of individuals who suffer from and who do not suffer from bad breath

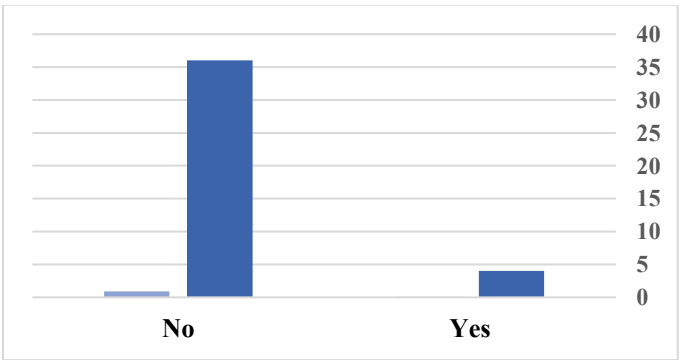

Figure 8: Distribution of individuals taking and not taking antibiotics

In this study, the data revealed that half of the of student's smoke, a high percentage compared to university expectations. This is a serious indicator of the harmful effects smoking can have on the mouth and body. This also highlights a possible link between smoking and the presence of Gram-positive bacteria in saliva. The findings revealed that all smokers had positive bacterial cultures (**Figure 9**), while no positive cases were recorded among non-smokers. The Chi-square test revealed a significant association between smoking and the prevalence of Gram-positive cocci ($p<0.001$; $\chi^2=36.1$), and Fisher's test confirmed a strong statistical significance ($p<0.00$). These results indicated a strong association between smoking and an increased likelihood of the presence of Gram-positive bacteria in the oral cavity in college students. The study indicated a strong association between smoking and an increased likelihood of the presence of Gram-positive bacteria in the oral cavity of medical technical college students. *Streptococcus spp* was present alone in the oral cavity of college students who smoked at a high rate (68.5%). *Staphylococcus spp* was not present alone in the oral cavity of smoking students. However, the presence of *Streptococcus spp* and *Staphylococcus spp* together in the oral cavity of college students was at a rate of 13.5% (**Figure 10**). These findings came in consistent with the previous studies [28-35].

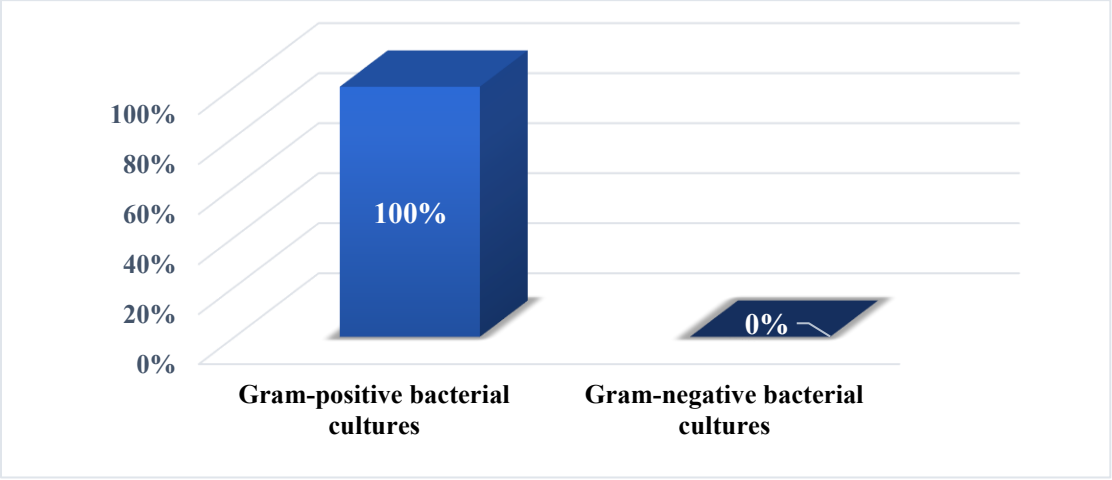

Figure 9: Positive and negative bacterial culture in Libyan smoking students

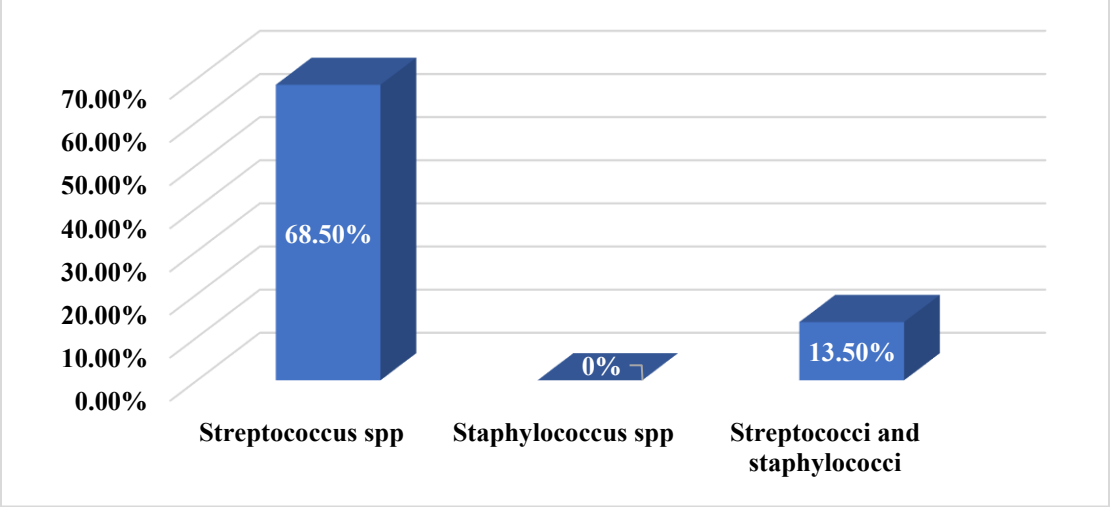

Figure 10: Distribution rate of *streptococci* and *staphylococci* among Libyan smoking students

Conclusion: The current study suggests a strong association between smoking and an increased likelihood of Gram-positive bacteria in the oral cavity of Libyan university students.

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